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TITLE: Role of NF-Kappa B Signaling in X-Box Binding Protein 1 (XBP1)-Mediated Antiestrogen Resistance in Breast Cancer

PRINCIPAL INVESTIGATOR: Dr. Rong Hu

CONTRACTING ORGANIZATION: Georgetown University
                                              Washington, DC 20057-0001

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Most breast cancer patients who undertake antiestrogen therapy eventually suffers from antiestrogen resistance. Understanding its molecular mechanism is essential for identifying potential targets to overcome antiestrogen resistance. XBP1-S, an important regulator of the unfolded protein response (UPR), is found highly expressed in antiestrogen resistant breast cancer cells and tissues. XBP1-S is believed to function as an important antiestrogen resistance mediator as overexpression of XBP1-S is sufficient to drive resistancy to antiestrogens in MCF7 cells. In this study, we aim to investigate the mechanism of XBP1-mediated antiestorgen resistance, specifically the involvement of NFkappaB signaling. We found that XBP1 regulates NFkappaB signaling in an ERalpha signaling dependent mechanism. We have examined the role of XBP1(U) and XBP1(S) in tumor development in vivo. We have injected nude mices with MCF7 cells that overexpress XBP1 and lacZ control cells. As expected, we observed enhanced growth in XBP1 overexpressed cells. We found that XBP1 overexpressed tumors are more resistant to tamoxifen treatment. NFkappaB inhibition with Parthenolide inhibit the tumor growth, and co-treatment of NFkappaB with tamoxifen further slows down the growth in XBP1 overexpressed tumors.

15. SUBJECT TERMS
None provided.

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a. REPORT	b. ABSTRACT	c. THIS PAGE			
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Table of Contents

I. Introduction:

Breast cancer is the most common cancer type among women, with 70% of the patients is estrogen receptor positive (ER+) and receives endocrine therapy (1). However, most patients develop antiestrogen resistance after long-term exposure. Overcome antiestrogen resistance will significantly improve the long-term treatment outcome of ER+ breast cancer patients. XBP1 is a known factor that promotes antiestrogen resistance in breast cancer (2-3). The overall hypothesis of this grant is that NFkB signaling is downstream of XBP1 pathway and is essential for XBP1 mediated antiestrogen resistance in breast cancer.

II. Research Accomplishment Body:

Aim: Determine the role of XBP1 in breast cancer antiestrogen resistance in vivo.

During the first two year of our study in this proposal, we have tested our hypothesis in the *in vitro* cell culture model. We have successfully showed that NFkB signaling is downstream of XBP1 pathway. We also confirmed that NFkB signaling is essential for the XBP1 mediated antiestrogen resistance in the *in vitro* cell culture model. In the past year, we have been trying to determine the role of XBP1 and its downstream NFkB in tumor growth and antiestrogen resistance *in vivo*.

Five-week old female ovariectomized NCR nu/nu mice were obtained from Taconic Farms, Inc. (Germantown, NY). Prior to the cell injection, a 60-day release 0.36mg 17-β estradiol pellet (Innovative Research of America, Sarasota, FL) was placed on the lower dorsal region of each nude mouse. $2x10^6$ cells resuspend in cell culture medium and equal volume of matrigel (BD Biosciences) were injected into each of the #4 mammary fat pads. After tumor size reaches 6mm in diameter, the mice were randomly grouped into four treatment groups: control (ethanol injection and regular diet); Parthenolide, tamoxifen, and both. 1mg/1kg Parthenolide were given via i.p injection twice a week and tamoxifen (400mg/kg Tamoxifen Citrate, Voigt Global Distribution) was given via diet. For all animals, tumor size was recorded twice a week until the sacrifice of animals.

In order to obtain statistically reliable data, we have to have at least 12 animals in each of the 12 treatment groups. However, because of the limited amount of animals we could handle in each experiment cycle, we have to break down the experiments into smaller groups. At the moment, we have completed part of our experiment, and the preliminary data are shown in Figure 1.

Similar to the pilot experiment we reported last year, we haven't observed any difference in take rate among three different cell lines. However, the tumor growth rate of XBP1 overexpressed tumors is higher than that of the LacZ control tumors. LacZ tumors are sensitive to tamoxifen treatment, with tumors stop growing shortly after tamoxifen diet were provided. As expected, XBP1-S and XBP1-U overexpressed cells are less sensitive to tamoxifen diet. In contrary, NFkB inhibitor Parthenolide was more effective in XBP1 overexpressed tumors. Furthermore, the presence of Parthnolide sensitizes the XBP1 overxpressed tumors to tamoxifen treatment. However, because the limited number of animals included in the experiments, a statistically significant data is not yet available. Additional experiments are needed for us to be able to draw

conclusions with the effects of NFkB inhibition with XBP1 overexpressed cells, both alone and in combination with tamoxifen.
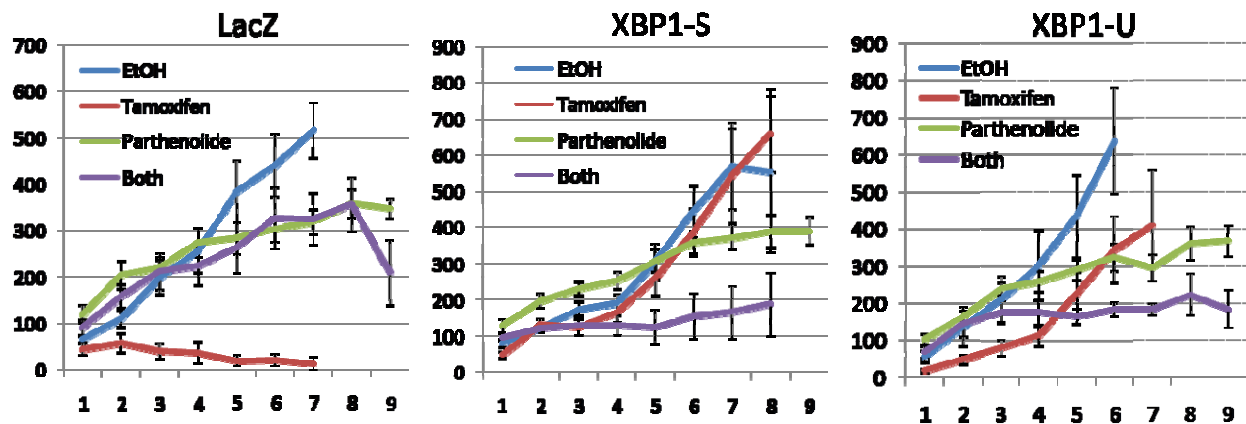

Figure 1. Tumor growth curve of XBP1- or lacZ- overexpressed tumor xenograft.

At the endpoint of the animal study, we have sacrificed animals and harvest the xenograft tumor for further analysis. XBP1 overexpressed tumors are often more vascularized than control cells, as shown by IHC staining with angiogenesis marker CD31 in last year's report. To further delineate the signaling pathway for the observed up-regulation of angiogenesis, we have examined HIF1alpha pathways that are known to play roles in angiogenesis (4). We found that HIF1alpha is highly induced by XBP1 overexpression. Interestingly, both spliced and unspliced form of XBP1 is able to induce HIF1alpha to a similar extent (Figure 2). This data suggest that the HIF1alpha pathway is activated and possibly responsible for the angiogenic effect observed in XBP1-overexpressed tumors.
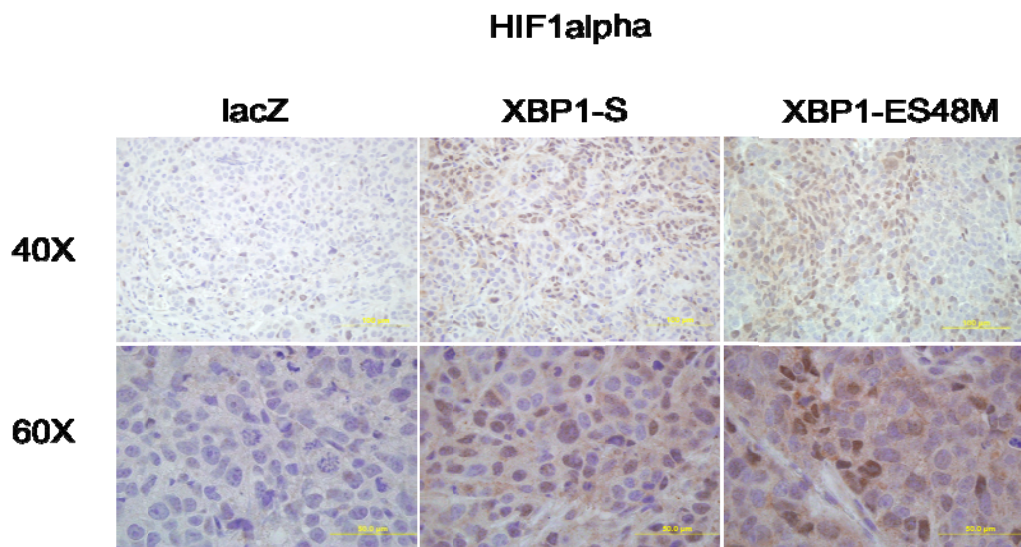

Figure 2. Representative pictures from tumor sample slide stained with HIF1alpha.

Another interesting observation is that IRE1α signaling, which is known to be upstream of XBP1 signaling, is also up-regulated in both XBP1-S and XBP1-U overexpressed cells (5). We have observed the phosophorylation of IRE1α induced by XBP1 overexpression in both the xenograft model and MCF7 cell culture (Figure 3). This result suggests a positive feedback loop between XBP1 overexpression and IRE1α signaling.
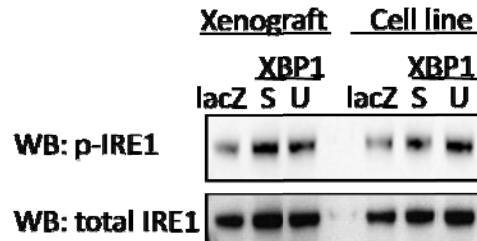

Figure 3. IRE1signaling is activated by XBP1 overexpression in both xenograft tumors and in MCF7 cells.

III. Key Research Accomplishments

XBP1-S and XBP1-U overexpression promote tumor growth *in vivo*.

XBP1-S and XBP1-U overexpressed tumor are sensitive, but lacZ overexpressed tumor is resistant to NFkB signaling inhibition.

XBP1-S cells are more resistant to tamoxifen in xenograft model.

XBP1-S overexpression induces HIF1alpha expression *in vivo*.

XBP1 overexpression induce a positive feedback activation of IRE1alpha signaling.

IV. Reportable Outcomes

Conference Abstract:

1) XBP1-NFkappaB signaling promotes anti-estrogen resistance in breast cancer animal model. Hu R., Zwart A., Warri A., Clarke R. Poster presentation at the 2013 Annual Meeting of the AACR.
2) Autophagy and unfolded protein response (UPR) signaling regulates progression of apoptosis in mammary gland involution. Cook K., Warri A., Hu R., Jin L., Zwart A., Pantoja D., Liu J., Finkel T., Clarke R. Poster presentation at the 2013 Annual Meeting of the AACR.
3) Estrogen receptor-alpha coordinates the unfolded protein response, autophagy, and reactive oxygen species generation to regulate breast cancer survival. Clarke P., Cook K., Hu R., Schwartz J., Abu-Asab M., Warri A., Clarke R. Poster presentation at the 2013 Annual Meeting of the AACR.

Manuscript in preparation:

Cook K., Clarke P., Parmar J., **Hu R.,** Schwartz-Roberts J., Abu-Asab M., Warri A., Baumann W., Clarke R. Knockdown of estrogen receptor-α induces autophagy and inhibits antiestrogen-mediated unfolded protein response activation promoting ROS-induced breast cancer cell death. Submitted to Molecular and Cellular Biology.

Hu R., Warri A., Zwart A., Tabor K., Shajahan A., Riggins R., Clarke R. NFkappaB Signaling up-regulation is required for XBP1 Mediated Antiestrogen Resistance in Breast Cancer Cells. Manuscript in preparation.

Warri A., Cook K., **Hu R.,** Jin L., Zwart A., Soto-Pantoja D., Liu J., Finkel T., Clarke R. Autophagy and Unfolded Protein Response (UPR) signaling regulate progression of mammary gland involution by restraining apopotis-driven irreversible changes. Manuscript in preparation.

V. Conclusions

From the third year of the funded research, we have made several positive findings. First, we showed that XBP1 overexpression is effective in promoting endocrine resistance *in vivo*. Interestingly, we showed that both XBP1-S and XBP1-U could promote endocrine resistance *in vivo*. Second, we determined the role of NFkB signaling and XBP1 in breast cancer in xenograft model. We found that NFkB signaling is essential for XBP1-overexpressed breast tumors. Inhibition of NFkB signaling alone is sufficient to slow down the growth of XBP1-overepxressed tumors. The combination of NFkB and Tamoxifen further inhibit the XBP1-overxpressed tumor growth. In the contrary, inhibition of NFkB signaling alone has limited effect in control lacZ tumors. However, we still need to repeat the experiments with additional animals to obtain statistically meaningful data. We have requested a one year no cost extension to DOD to continue our experiments.

VI. References

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2. Gomez, B.P., Riggins, R.B., Shajahan, A.N., Klimach, U., Wang, A., Crawford, A.C., Zhu, Y., Zwart,A., Wang, M., and Clarke, R., *Human X-box binding protein-1 confers both estrogen independence andantiestrogen resistance in breast cancer cell lines.* FASEB J., 2007. **21**(14): p. 4013-27.

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4. Yang Y., Sun M., Wang L., Jiao B. *HIFs, angiogenesis, and cancer.* J. Cell Biochem, 2013 114(5): 967-74.

5. Calfon M., Zeng H., Urano F., Till JH., Hubbard SR., Harding HP., Clark SG., Ron D., IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. Nature 2002 415: 92-6.

VII. Appendices

1. Abstract presented at the 2013 Annual Meeting of the AACR (3 page).

2. Current *curriculum vitae* (2 pages).

Late-Breaking Poster Presentations - Unfolded Protein Response

Abstract LB-254: XBP1-NFkappaB signaling promotes antiestrogen resistance in breast cancer animal model.

Rong Hu, Alan Zwart, Anni Warri, and Robert Clarke

Georgetown Lombardi Comp. Cancer Ctr., Arlington, VA.

There are nearly 3 million breast cancer survivors in the United States, with more than 230,000 women being newly diagnosed with invasive breast cancer each year. Of total breast cancer patients, around 70 percent are estrogen receptor (ER) positive and often receive endocrine therapy. Endocrine resistance, either de novo or acquired, is a major hurdle for treating ER positive breast cancer patients. Unfolded protein response (UPR) is known to be activated and promote endocrine resistance in breast cancer cells. As an essential component of URP signaling, X-box binding protein 1 (XBP1) was shown up-regulated in endocrine resistant breast cancer. XBP1 was demonstrated as an important mediator for endocrine resistance in vitro cell culture model in our previous findings. In this study, the effect of XBP1 in endocrine resistance in animal model was examined. XBP1 contains two alternative splice variants: XBP1(S) and XBP1(U). In order to differentiate the role of each variant, we generated XBP1 mutant constructs that overexpress specifically only either the XBP1(S) or XBP1(U).

The XBP1 over-expressing human breast cancer cells have been injected into the mammary fat pad of female nude mice. The preliminary data shows that the over-expression of both XBP1 splice variants promotes tumor growth in vivo. More importantly, tumors with over-expressed XBP1 show less sensitivity to tamoxifen treatment. Our previous finding revealed that NFkappaB signaling is regulated by XBP1 and required for antiestrogen resistance in cultured cells. In the current study, we have treated XBP1-overexpressed breast tumor bearing animals with NFkappaB inhibitor Parthenolide, and find that it enhances tamoxifen sensitivity. In conclusion, our data demonstrate that XBP1-NFkappaB signaling plays an important role in antiestrogen resistance in vivo.

Poster Presentations - Autophagy and Cell Death

Abstract 1667: Autophagy and unfolded protein response (UPR) signaling regulates progression of apoptosis in mammary gland involution.

Katherine L. Cook[1], Anni Warri[1]. Rong Hu[1], Lu Jin[1], Alan Zwart[1], David R. Soto Pantoja[2], Jie Liu[2], Toren Finkel[2], and Robert Clarke[1]

[1]Georgetown University, Washington, DC;
[2]National Institutes of Health, Bethesda, MD.

Progression of local benign mammary lesions to invasive breast cancer is known to involve processes that are necessary for normal mammary gland development. Post-natal mammary gland is capable of undergoing repeated cycles of proliferation and cell death, most strikingly when fully differentiated (lactating) gland degenerates to a pre-lactation state during involution. Involution involves extensive cell death and tissue remodeling, which may temporarily increase breast cancer risk. Accumulation of milk proteins in the secretory epithelium creates the stress signal that triggers involution; however it is largely unknown whether the well-defined stress pathways, UPR and autophagy, are involved breast involution regulation. Using published gene expression array datasets from different phases of mouse mammary gland development we demonstrate that UPR, autophagy and apoptosis genes grouped in separate clusters. Next, in time-course experiments we show that autophagy and UPR signaling are tightly co-regulated during mammary involution. Early UPR signaling events such as GRP78 and phospho-eIF2α were elevated during early involution, i.e. the first 24 - 48 hours (h; reversible phase), while late UPR signaling events such as ATF4 and CHOP were upregulated during 72 h - 7 days of involution. An increase in AMPK was observed during early involution potentially preventing TORC1-mediated autophagy inhibition. Correlating with these observations, increased LC3-GFP punctate and decreased p62 expression were observed at 24 h when compared to 72 h post weaning, suggesting increased autophagy. Apoptosis was increased 48-72 h, peaking at 72 h involution, as measured by TUNEL. Increased apoptotic markers, such as cleaved caspase-7 and cleaved PARP were observed during these time points, with increased antiapoptotic BCL2 family members (BCL-XL and BCL-W) expressed from 24-48 h. To confirm the causal and inverse relationship between autophagy and apoptosis signaling, we performed involution time-course experiments using both low-dose drug interventions and an autophagy-related gene 7 (ATG7) deletion mouse model. Inhibition of autophagy by chloroquine, or genetic deletion of one ATG7 allele, enhanced the progression of mammary involution into an irreversible phase, characterized by earlier induction of apoptosis. In contrast, stimulation of autophagy by low dose tunicamycin treatment reduced apoptosis and extended the reversible phase of mammary involution by sustaining the secretory epithelium, suggesting a possible therapeutic use of autophagy stimulators in promoting lactation and nursing. Taken together these data indicate that UPR and autophagy play a key role in the regulation between survival and apoptosis during normal mammary gland involution and homeostasis, and, thus, if deregulated could contribute to breast cancer progression.

Citation Format: Katherine L. Cook, Anni Warri, Rong Hu, Lu Jin, Alan Zwart, David R. Soto Pantoja, Jie Liu, Toren Finkel, Robert Clarke. Autophagy and unfolded protein response (UPR) signaling regulates progression of apoptosis in mammary gland involution. [abstract]. In: Proceedings of the 104th Annual Meeting of the American Association for Cancer Research; 2013 Apr 6-10; Washington, DC. Philadelphia (PA): AACR; Cancer Res 2013;73(8 Suppl):Abstract nr 1667. doi:10.1158/1538-7445.AM2013-1667

Poster Presentations - Cancer Mechanisms and Medicine 2

Abstract 4046: Estrogen receptor-α coordinates the unfolded protein response,autophagy, and reactive oxygen species generation to regulate breast cancer survival.

Pamela A. Clarke[1], Katherine Cook[1], Rong Hu[1], Jessica L. Schwartz[1], Mones Abu-Asab[2], Anni Warri[1], and Robert Clarke[1]

[1]Georgetown University, Washington, DC;
[2]National Institutes of Health, Bethesda, MD.

Of the almost 240,000 new breast cancers diagnosed in 2011 within the USA, approximately 70% of these cases express the estrogen receptor-α (ER). ER targeted therapies such as tamoxifen (TAM) or faslodex (ICI) are often used to treat this breast cancer subtype. However, the curative potential of these interventions is frequently limited in patients due to resistance. Understanding how antiestrogen resistance occurs could lead to improved approaches to eradicate ER+ breast cancers. Increased unfolded protein response (UPR, an endoplasmic reticulum stress pathway) or autophagy (a "self" eating process used to clear dysfunctional protein or organelles) was previously shown to promote antiestrogen resistance in ER+ breast cancer. Using the antiestrogen sensitive MCF7-LCC1 (LCC1) and resistant MCF7-LCC9 (LCC9) cell lines, we determined the effect of antiestrogens and ERα on autophagy and UPR signaling. Suprisingly, ablation of ERα through RNAi potentiated LCC1 to antiestrogen-mediated cell death and resensitized LCC9 cells to endocrine therapy. While ICI stimulated both autophagy and UPR signaling, RNAi knockdown of ERα in LCC1 and LCC9 cells inhibited ICI-mediated UPR signaling and concurrently stimulated autophagy. Furthermore, we showed that ERα knockdown stimulated ROS production and potentiated the cell death response to antiestrogen therapy. ERα knockdown, but not ICI treatment, reduced nuclear Nrf2 (a UPR-induced antioxidant signaling protein) and increased cytosolic KEAP1 (an inhibitor of Nrf2), which may lead to the observed increase in ROS production. Autophagy inhibition through ATG7 silencing increases cell death and apoptosis in LCC1 ERα knockdown cells, showing that autophagy induction by antiestrogens is prosurvival in this context. These data suggests that UPR and autophagy stimulated by antiestrogen therapy results from two different mechanisms of ER regulation. We show that antiestrogens promote the accumulation of ERα in the cytosol that is associated with increased UPR signaling, while the inhibition of ERα promotes prosurvival autophagy. The inherent stimulation of resistance mechanism signaling through antiestrogen treatment suggests that combining autophagy or UPR inhibitors with anti-estrogens may greatly reduce the development of acquired antiestrogen resistance in breast cancer.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel on page 1 of the Detailed Cost Estimate form for the initial budget period.

NAME	POSITION TITLE
Rong Hu	Post-doctoral Fellow

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Nanchang University	B.S.	1999-2003	Biotechnology
University of Leicester	M.Sc.	2003-2004	Molecular Genetics
SUNY-Albany		2004-2005	Molecular Biology
Albany Medical college		2005-2008	Cancer Biology
Thomas Jefferson University	Ph.D	2008-2009	Genetics
Georgetown University Medical Center	Post-Doc	2010-Present	Breast Cancer

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List in chronological order the titles, all authors, and complete references to all publications during the past 3 years and to representative earlier publications pertinent to this application. If the list of publications in the last 3 years exceeds 2 pages, select the most pertinent publications. PAGE LIMITATIONS APPLY. DO NOT EXCEED 4 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INDIVIDUAL.

Research Experiences:

09/2003-08/2004:   MSc. In Molecular Genetics, University of Leicester, Leicester, UK
Thesis: Mechanism of Translational Selection of mRNAs during Apoptosis
Mentor: Dr. Martin Bushell

06/2005-11/2009:   Ph.D in Genetics, Albany Medical College and Thomas Jefferson University
Thesis: F-box proteins and co-factors of SCF E3 ubiquitin ligases in melanoma.
Mentor: Dr. Andrew Aplin

01/2010-Present: Post-doctoral Fellow, Georgetown University
Research: Role of UPR signaling in endocrine resistance in breast cancer
Mentor: Dr. Robert Clarke

Publications and Manuscript in preparation:

Bhatt KV., **Hu. R.,** Spofford LS., Aplin AE. (2007) Mutant B-RAF signaling and cyclin D1 regulate Cks1/S-phase kinase-associated protein 2-mediated degradation of p27Kip1 in human melanoma cells. Oncogene. 26(7): 1056-1066.

Hu R. and Aplin AE. (2008) Skp2 regulates G2/M progression in a p53-dependent manner. Mol. Biol. Cell. 19(11): 4620-4610.

Hu R. and Aplin AE. (2009) αB-crystallin is mutant B-RAF regulated and contributes to cyclin D1 turnover in melanocytic cells. Pigment Cell & Melanoma Research. 2010 Jan 27. Epub ahead of print.

Clarke R., Cook KL., **Hu R.,** Facey COB., Tavassoly I., Schwartz J., Baumann WT., Tyson JJ., Xuan J., Wang Y., Warri A., Shajahan AN. (2012) Endoplasmic reticulum stress, the unfolded protein response, autophagy, and the integrated regulation of breast cancer cell fate. Cancer Res. 72: 1321-1331.

Harini SA., Bouker KB., Cook KL., Facey COB., **Hu R.,** Schwartz J., Shajahan AN., Hilakivi-Clarke L., Clarke R. 2012 Interaction of dietary polyphenols with molecular signaling pathways of antiestrogen resistance: possible role in breast cancer recurrence. Hormones in Normal and Cancerous Breast: Basic and Clinical Applications. 9: 127-141.

Cook K., Clarke P., Parmar J., **Hu R.,** Schwartz-Roberts J., Abu-Asab M., Warri A., Baumann W., Clarke R. Knockdown of estrogen receptor-α induces autophagy and inhibits antiestrogen-mediated unfolded protein response activation promoting ROS-induced breast cancer cell death. Submitted to Molecular and Cellular Biology.

Hu R., Warri A., Zwart A., Tabor K., Shajahan A., Riggins R., Clarke R. NFkappaB Signaling up-regulation is required for XBP1 Mediated Antiestrogen Resistance in Breast Cancer Cells. Manuscript in preparation.

Warri A., Cook K., **Hu R.,** Jin L., Zwart A., Soto-Pantoja D., Liu J., Finkel T., Clarke R. Autophagy and Unfolded Protein Response (UPR) signaling regulate progression of mammary gland involution by restraining apopotis-driven irreversible changes.   Manuscript in preparation.

Funding:

Pre-doctoral Fellowship, National Cancer Center, 09/2007-09/2009
Skp2 regulation of melanoma cell proliferation: mechanism and role in a skin-like microenvironment

Post-doctoral Fellowship, Department of Defense, 09/2010-10/2013
Role of NFkB signaling in X-box binding protein 1(XBP1)-mediated antiestrogen resistance in breast cancer

Conferences:

Nov. 2007   International Melanoma Congress   New York, NY
Poster: p53 and cyclin E1-dependent effects of Skp2 on melanoma cell cycle

Apr. 2008   AACR Annual Meeting   San Diego, CA
Poster: Skp2 regulates G2/M progression in a p53-dependent manner

Sep. 2009   PanAmerican Society for Pigment Cell Research Annual Meeting Memphis, TN

Poster: F-box protein co-factor Cks1 and αB-crystallin: B-RAF regulation and roles in melanoma cell cycle progression

Apr. 2011   AACR Annual Meeting   Orlando, FL
Poster: XBP1 regulates NFkB signaling in Antiestrogen resistant breast cancer cells

Apr. 2011   Experimental Biology Annual Meeting   Washington, DC
Poster: IRF1 promotes antiestrogen sensitivity by regulating Bik expression in breast cancer cells

Aug. 2011   Era of Hope Breast Cancer Meeting   Orlando, FL
Poster: NFkB Signaling is required for XBP1-mediated antiestrogen resistance in breast cancer

Apr. 2013   AACR Annual Meeting   Washington, DC
Poster: XBP1-NFkappaB signaling promotes anti-estrogen resistance in breast cancer animal model
Poster: Estrogen receptor-alpha coordinates the unfolded protein response, autophagy, and reactive oxygen species generation to regulate breast cancer survival.
Poster: Autophagy and unfolded protein response (UPR) signaling regulates progression of apoptosis in mammary gland involution.

Awards and Honors:

Distinguished Student Award, Nanchang University, 2002, 2003

Student Scholarship, Nanchang University, 2000, 2001, 2002, 2003

Distinguished Student, University of Leicester, 2004

Richard A. Miller Alumni Prize, Albany Medical College, 2008

Dean's Excellence in Extramural Research Activities, Albany Medical College, 2008

Travel Award for 15th Annual Meeting of the PanAmerican Society for Pigment Cell Research, 2009

1st place in Poster Award for 15th Annual Meeting of the PanAmerican Society for Pigment Cell Research, 2009

Mentoring:

Irene Thung: Irene was a medical student at Georgetown University who performed her research intern in Dr. Clarke's laboratory. In summer 2010, I worked with Irene toward the completion of her research project, which focuses on understanding the effects of Akt/mTOR dual inhibitors in antiestrogen-resistant breast cancer.

Ahreej Eltayeb: Ahreej was a graduate student at George Washington University who performed her research in Dr. Clarke's laboratory. From November 2010, I worked with Ahreej toward investigating the role of XBP1 splicing in antiestrogen resistance in breast cancer. I assisted Ahreej in crafting her research plan for the supplemental RO1 award that she received. Ahreej is now working as a full-time technician in Dr. Clarke's lab.

Katie Tabor: Katie is a medical student at Georgetown University working in Dr. Clarke's laboratory for her independent study project. From summer 2011, I worked with Katie on her project on understanding the role of unspliced form of XBP1 (XBP1-U) in apoptosis and autophagy in breast cancer.

Amanda Rosen: Amanda is a senior student at Georgetown University working towards her Bachelors' degree. She is working in Dr. Clarke's laboratory for undergraduate thesis. From summer 2012, I worked with Amanda on her project on understanding the mechanism of up-regulated XBP1 in LCC9 antiestrogen resistant breast cancer cells.